DIPHTHERIA BACILLI FROM POSTOPERATIVE EMPYEMA WOUNDS

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On March 24, 1918, an organism corresponding morphologically to the diphtheria bacillus was found in a culture from a healing postoperative empyema wound. Cases showing this organism increased in number until a short time later sixty of the empyema wounds were infected. The wards were immediately quarantined and all possible care was taken to prevent further spread of the infection. In spite of vigorous treatment positive cultures were obtained after long intervals and in many cases even after 5 months. Because confusing reports have been made of the corynebacteria found in different parts of the body and because of the uncertainty as to the danger attendant with this particular infection an attempt was made to determine the exact nature of the organisms, their morphology, cultural characteristics, virulence for guinea-pigs and specific serum reactions. Efforts were made also to find some satisfactory method of disinfecting the wounds.

Twenty-six strains from infected wounds and five from throats including three positive controls from Hachita, N. M., were isolated in pure culture and studied, with results as follows:

Morphology.—All primary cultures were made on Loeffler's serum, incubated for 18-20 hours at 37 C., and smears stained with Loeffler's alkaline methylene blue and Neisser's method. Wesbrook's classification[1] was followed as a standard; only granular organisms corresponding to types A, C and D were reported as positive, while types A_1, C_1, D_1 and A_2, C_2 and D_2 were noted but disregarded in making routine reports. Practically all positive cultures contained the subtypes in varying numbers and, as found by others, these were present in increasing numbers after the infection had existed for a long period of time. Whether this was due to an inhibition of the granular forms and overgrowth by the solid and barred types, or whether they were involution or "degenerative" forms, is undecided. It was the rule to obtain a number of types together, even from pure cultures which had been carefully picked from single isolated colonies and transferred many times. Once the incubator temperature rose to 42 C. during the night, following which there

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[1] Wesbrook, F. F., Wilson, L. B., and McDaniel, O.: Transactions of the Association of American Physicians, Vol. 25, 1900, p. 198.

was a marked increase in the number of short forms in all cultures. The same phenomenon has been observed by Kolmer and others. All organisms were gram-positive, but easily decolorized. Stained with carbolfuchsin and decolorized with 70% acid alcohol 30 seconds, none were acid fast.

Isolation.—Positive cultures were planted on serum agar, ½ c c horse serum to 5 c c meat infusion agar, and incubated 24 hours. Smears from suspicious colonies were stained and, if positive, one colony was fished and planted on half of a second serum agar plate.

From this second plate, after 24 hours' incubation, colonies positive on smear were planted on Loeffler's serum. These cultures were examined after 18-24 hours' incubation and were accepted only if they were pure Klebs-Loeffler bacilli and showed a few, at least, of Wesbrook's A, C, or D types, but almost invariably the subtypes were present in varying numbers. Broth cultures (meat extract) were made for the inoculation of guinea-pigs from these slants.

Cultural Characteristics.—Although differing in some instances, most observers agree that the true virulent diphtheria bacillus produces fermentation of the monosaccharids dextrose and dextrin; and fails to do so in the case of the disaccharid saccharose.

Fox[2] states of pseudodiphtheria bacilli that "they are able to ferment dextrose, saccharose and maltose; while true diphtheria bacilli acidify dextrose, dextrin and maltose, but never saccharose." Cary[3] concluded "that the monosaccharids are fermentable by true diphtheria bacilli, but that the higher sugars are fermented less characteristically and less uniformly." However, in a few instances, true toxic diphtheria bacilli have been observed which were able to produce acid in saccharose medium and Graham-Smith reported a whole epidemic in 1908 produced by saccharose-fermenting, diphtheria bacilli.

Tubes containing 1% each of saccharose, dextrose, dextrin, galactose, maltose, lactose and glycerol in sugar-free beef-infusion broth were inoculated with pure cultures from each strain. Andrade's solution was used as an indicator and four readings were made at 2-day intervals. If readings had been recorded after a shorter period of time a number of reactions would have been missed, since some of the strains were slower than others in the production of acid. Smears were made and Loeffler's slants inoculated to determine vitality and purity of cultures. Uninoculated control tubes were incubated for the same period of time as the carbohydrates tested. As shown in table 1, the strains examined fell into 6 groups.

The strains in group 1 fermented all the carbohydrates excepting saccharose and glycerol. These strains included cultures from the empyema ward. All of the positive control cultures from clinical cases of pharyngeal diphtheria (Hachita, N. M.) likewise arranged themselves in this group. The virulence of these strains varied considerably; two of the wound cultures, one throat culture (clinical diphtheria, empyema ward) and one control were "very virulent"; one culture from throat of nurse on duty in an empyema ward was of intermediate virulence; one wound culture and one positive control culture from a clinical case (Hachita, N. M.) were of slight virulence. The other positive controls from throat diphtheria were virulent for guinea-pigs. These strains were all recovered at necropsy after which there was no change in their fermentation reactions.

Of the eighteen strains in group 2 fermenting saccharose, dextrose and galactose, and failing to ferment dextrin, maltose, lactose and glycerol, none

[2] Arch. Int. Med., Sept., 1915.

[3] Jour. Inf. Dic., 1917, 20, p. 244.

were of remarkable virulence. Three pigs died 9 days, and one each 15, 16, 20, 22 and 32 days, respectively, after inoculation, and ten are still living. Only one strain was recovered (death after 16 days) but the organism isolated at necropsy showed a change of its fermentation reactions.

None of the strains in groups 3, 4, 5, 6 fermented saccharose or proved virulent for guinea-pigs.

Growth on coagulated blood serum was moist and confluent in all but four strains; 19 were grayish-white in color, two were colorless and ten showed a slight pinkish tinge. All of the virulent strains were moist, confluent, grayish-white and failed to show any proteolytic activity.

TABLE 1

FERMENTATIONS

	Source of Cultures	Virulence for Guinea-Pigs	Saccharose	Dextrose	Galactose	Dextrin	Maltose	Lactose	Glycerol
I	2 wound cultures 1 throat, empyema 1 throat, diphtheric (Hachata, "A") 1 throat, nurse, diphtheric, empyema ward 1 wound culture 1 throat, diphtheric (Hachita, "B") 1 throat, diphtheric patient "B" control	Very virulent Intermediate virulence Slight virulence Low virulence	0	+	+	+	+	+	0
II	8 wound cultures 10 wound cultures	Slight virulence Avirulent	+	+	+	0	+	0	0
III	2 wound cultures	Avirulent	+	+	+	0	+	0	0
IV	1 wound culture	Avirulent (Xerosis?)	+	+	+	0	+	0	+
V	1 wound culture	Avirulent	+	+	0	0	0	0	+
VI	1 wound culture	Avirulent (Hoagi?)	+	+	0	0	0	0	0

Total strains from empyema ward, 28.
Virulent strains from empyema ward, 5 or 17.8%.
Wound strains from empyema ward, 26.
Virulent wound strains from empyema ward, 3 or 11.5%.
Throat strains from empyema ward, 2.
Virulent throat strains from empyema ward, 2 or 100%.
Controls: Clinical throat strains (Hachita) all saccharose and glycerol negatives, 3. Very virulent, 1; slightly virulent, 1; avirulent, 1.

The formation of a pellicle on broth cultures was not constant and occurred only with the less virulent strains. Observations were made at the end of 24 and 48 hours, using both plain and glucose broth cultures.

For the determination of hemolysis a small loop of culture was mixed in 5 c c of sterile normal salt solution, from which one loopful was added to 5 c c melted agar with 0.5 c c defibrinated horse blood. This was then poured into sterile Petri dishes and incubated. Examination for hemolysis was made with the microscope and was recorded as positive only when the red cell outlines had completely disappeared. Four virulent and two avirulent strains produced a definite zone of hemolysis.

All the strains failed to produce indol in Dunham's peptone medium. Titrations were made on the 4th day.

Seventeen cultures including all the virulent strains gave a scarcely perceptible pink color in litmus milk, while in the remaining fourteen no change was detected.

Virulence.—For Man: In two early cases the patients died; one (throat ++) died March 19, and another who gave positive cultures from wound, nose and throat April 18, died April 20. Four cases developed paralytic conditions even after administration of antitoxin. Unfortunately the cultures from the fatal cases were lost. A nurse developed a typical pharyngeal diphtheria while on duty in the quarantined empyema ward, the organisms from the throat being of intermediate virulence for guinea-pigs.

For Guinea-Pigs: Guinea-pigs weighing less than 300 gm. were inoculated subcutaneously with 0.5% of their body weight, reckoned in cubic centimeters, of a 72-hour broth culture. A control pig was given the same proportional dose, after having had 24 hours previously, 100 units of diphtheria antitoxin intraperitoneally. All animals were examined as soon after death as possible and cultures made from subcutaneous tissues at the site of inoculation, from peritoneal or pleural exudates, when present, and from the heart blood. The control pigs were protected by the diphtheria antitoxin which they received.

Three strains from the empyema ward produced death of guinea-pig in one to two days; two of these strains were isolated from wounds and one from an apparently normal throat. One of the positive control throat cultures (Hachita, N. M.) was also very virulent. The organisms were recovered in all these cases.

One culture, which was obtained from the throat of a nurse 'C' who developed pharyngeal diphtheria while on duty in the quarantined empyema ward, killed a guinea-pig in four days.

Of three wound cultures which produced death of guinea-pig in 6-10 days, two fermented saccharose, the third failed to do so. This was also true of another positive control culture (Hachita, N. M.).

One culture, which produced only a local lesion, was obtained from the throat of a patient who was admitted to the hospital with 'laryngeal diphtheria'. This organism failed to ferment saccharose.

Microscopic sections through the abdominal wall at the site of the inoculation showed ulceration of the surface. The bands of connective tissue in the subcutaneous fatty layer and the fascia were edematous and infiltrated with polymorphonuclear leukocytes and corpuscles. The muscle fibers adjacent to the fascia were infiltrated also. The infection did not penetrate the muscle and the peritoneum was normal. Stained by Weigert's method great quantities of gram-positive bacilli, some stained irregularly, were seen.

Twenty cultures produced no noticeable effect on guinea-pigs. These were all saccharose fermenters.

Specific Serum Reactions.—Tests made to determine the presence of complement fixing, precipitating and agglutinating antibodies in the serum of the infected individuals all failed to give definite results.

Blood cultures were made in all cases with negative results.

In addition, comparative studies were made of cultures from discharging empyema wounds of 30 patients in the base hospital at Camp Travis. None of these cultures showed diphtheria bacilli, but three gave B. hoffmannii.

Treatment.—All patients were isolated, quarantined and given diphtheria antitoxin in large amounts when the first positive culture was reported. Schick

tests were not made until after the initial antitoxin inoculations. The reactions were all negative, as one would expect, and when repeated, after an interval of several months, the results were similar. Dichloramin-T solution was used locally but did not prove of value. Later the cases were divided into four groups each of which was treated differently: Group 1 received daily irrigations with Dakin's solution; group 2, wounds were cleaned and swabbed with Mandell's solution daily; group 3, wounds were irrigated daily with diphtheria antitoxin; while the group 4 wounds were cleansed and exposed to direct sunlight as long as possible daily. Cultures made every other day over a period of several months failed to show any marked differences in the results obtained with these various methods of treatment. The decrease in positive cases was slow, and as a rule the carrier state continued until the operative wound was completely healed.

It was noticed that whenever the wounds were curetted, the total number of positive cultures were greatly increased, including many cases which had been considered negative for some time. This probably explains the failure of the therapeutic measures tried as it seems to indicate that many of the diphtheria bacilli were deeply buried between the crevices or within the granulation tissue and for this reason were not reached by the antiseptics used.

SUMMARY

The organisms isolated from empyema wounds were morphologically true diphtheria bacilli.

Of the strains isolated, 17.8% were virulent for guinea-pigs, and all of these strains failed to produce acid when grown in saccharose broth cultures for 8 days. The degree of virulence among saccharose negative strains was variable.

The morphological characteristics presented by both virulent and avirulent strains were the same. All cultures contained a mixture of Wesbrook's type A, C and D, with the subtypes.

There was no evidence of the development of specific agglutinins, precipitins or complement fixing substances for diphtheria bacilli (virulent or avirulent) in the serum of the infected individual.

Apparently there was no invasion of the blood stream by the diphtheria bacilli in the wounds.

All methods of treatment tried proved unsatisfactory, due probably to the growth of bacilli deep in the wound granulations. As a rule the carrier state continued until complete healing of the wounds had taken place.